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Patentanmeldung Nr. Patent application No. Demande de brevet n°

02008712.8

Der Präsident des Europäischen Patentamts;
Im Auftrag

For the President of the European Patent Office

Le Président de l'Office européen des brevets
p.o.

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Anmeldung Nr:
Application no.: 02008712.8
Demande no:

Anmeldetag:
Date of filing: 18.04.02
Date de dépôt:

Anmelder/Applicant(s)/Demandeur(s):

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Bezeichnung der Erfindung/Title of the invention/Titre de l'invention:
(Falls die Bezeichnung der Erfindung nicht angegeben ist, siehe Beschreibung.
If no title is shown please refer to the description.
Si aucun titre n'est indiqué se referer à la description.)

Modified factor VIII

In Anspruch genommene Priorität(en) / Priority(ies) claimed /Priorité(s) revendiquée(s)
Staat/Tag/Aktenzeichen/State/Date/File no./Pays/Date/Numéro de dépôt:

Internationale Patentklassifikation/International Patent Classification/
Classification internationale des brevets:

C07K14/00

Am Anmeldetag benannte Vertragstaaten/Contracting states designated at date of filing/Etats contractants désignées lors du dépôt:

AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE TR

MODIFIED FACTOR VIII

FIELD OF THE INVENTION

The present invention relates to polypeptides to be administered especially to humans and in particular for therapeutic use. The polypeptides are modified polypeptides whereby the modification results in a reduced propensity for the polypeptide to elicit an immune response upon administration to the human subject. The invention in particular relates to the modification of human Factor VIII (FVIII) to result in FVIII proteins that are substantially non-immunogenic or less immunogenic than any non-modified counterpart when used *in vivo*. The invention relates furthermore to T-cell epitope peptides derived from said non-modified protein by means of which it is possible to create modified FVIII variants with reduced immunogenicity.

BACKGROUND OF THE INVENTION

There are many instances whereby the efficacy of a therapeutic protein is limited by an unwanted immune reaction to the therapeutic protein. Several mouse monoclonal antibodies have shown promise as therapies in a number of human disease settings but in certain cases have failed due to the induction of significant degrees of a human anti-murine antibody (HAMA) response [Schroff, R. W. et al (1985) *Cancer Res.* 45: 879-885; Shawler, D.L. et al (1985) *J. Immunol.* 135: 1530-1535]. For monoclonal antibodies, a number of techniques have been developed in attempt to reduce the HAMA response [WO 89/09622; EP 0239400; EP 0438310; WO 91/06667]. These recombinant DNA approaches have generally reduced the mouse genetic information in the final antibody construct whilst increasing the human genetic information in the final construct. Notwithstanding, the resultant "humanized" antibodies have, in several cases, still elicited an immune response in patients [Issacs J.D. (1990) *Sem. Immunol.* 2: 449, 456; Rebello, P.R. et al (1999) *Transplantation* 68: 1417-1420].

Antibodies are not the only class of polypeptide molecule administered as a therapeutic agent against which an immune response may be mounted. Even proteins of human origin and with the same amino acid sequences as occur within humans can still induce an immune response in humans. NoTable

examples include the therapeutic use of granulocyte-macrophage colony stimulating factor [Wadhwa, M. et al (1999) *Clin. Cancer Res.* 5: 1353-1361] and interferon alpha 2 [Russo, D. et al (1996) *Bri. J. Haem.* 94: 300-305; Stein, R. et al (1988) *New Engl. J. Med.* 318: 1409-1413] amongst others.

A principal factor in the induction of an immune response is the presence within the protein of peptides that can stimulate the activity of T-cells via presentation on MHC class II molecules, so-called "T-cell epitopes". Such potential T-cell epitopes are commonly defined as any amino acid residue sequence with the ability to bind to MHC Class II molecules. Such T-cell epitopes can be measured to establish MHC binding. Implicitly, a "T-cell epitope" means an epitope which when bound to MHC molecules can be recognized by a T-cell receptor (TCR), and which can, at least in principle, cause the activation of these T-cells by engaging a TCR to promote a T-cell response. It is, however, usually understood that certain peptides which are found to bind to MHC Class II molecules may be retained in a protein sequence because such peptides are recognized as "self" within the organism into which the final protein is administered.

It is known, that certain of these T-cell epitope peptides can be released during the degradation of peptides, polypeptides or proteins within cells and subsequently be presented by molecules of the major histocompatability complex (MHC) in order to trigger the activation of T-cells. For peptides presented by MHC Class II, such activation of T-cells can then give rise, for example, to an antibody response by direct stimulation of B-cells to produce such antibodies.

MHC Class II molecules are a group of highly polymorphic proteins which play a central role in helper T-cell selection and activation. The human leukocyte antigen group DR (HLA-DR) are the predominant isotype of this group of proteins and are the major focus of the present invention. However, isotypes HLA-DQ and HLA-DP perform similar functions, hence the present invention is equally applicable to these. The MHC class II DR molecule is made of an alpha and a beta chain which insert at their C-termini through the cell membrane. Each hetero-dimer possesses a ligand binding domain which binds to peptides varying between 9 and 20 amino acids in length, although the binding groove can

accommodate a maximum of 11 amino acids. The ligand binding domain is comprised of amino acids 1 to 85 of the alpha chain, and amino acids 1 to 94 of the beta chain. DQ molecules have recently been shown to have an homologous structure and the DP family proteins are also expected to be very similar. In humans approximately 70 different allotypes of the DR isotype are known, for DQ there are 30 different allotypes and for DP 47 different allotypes are known. Each individual bears two to four DR alleles, two DQ and two DP alleles. The structure of a number of DR molecules has been solved and such structures point to an open-ended peptide binding groove with a number of hydrophobic pockets which engage hydrophobic residues (pocket residues) of the peptide [Brown et al *Nature* (1993) 364: 33; Stern et al (1994) *Nature* 368: 215]. Polymorphism identifying the different allotypes of class II molecule contributes to a wide diversity of different binding surfaces for peptides within the peptide binding grove and at the population level ensures maximal flexibility with regard to the ability to recognize foreign proteins and mount an immune response to pathogenic organisms.

There is a considerable amount of polymorphism within the ligand binding domain with distinct "families" within different geographical populations and ethnic groups. This polymorphism affects the binding characteristics of the peptide binding domain, thus different "families" of DR molecules will have specificities for peptides with different sequence properties, although there may be some overlap. This specificity determines recognition of Th-cell epitopes (Class II T-cell response) which are ultimately responsible for driving the antibody response to B-cell epitopes present on the same protein from which the Th-cell epitope is derived. Thus, the immune response to a protein in an individual is heavily influenced by T-cell epitope recognition which is a function of the peptide binding specificity of that individual's HLA-DR allotype. Therefore, in order to identify T-cell epitopes within a protein or peptide in the context of a global population, it is desirable to consider the binding properties of as diverse a set of HLA-DR allotypes as possible, thus covering as high a percentage of the world population as possible.

An immune response to a therapeutic protein proceeds via the MHC class II peptide presentation pathway. Here exogenous proteins are engulfed and

processed for presentation in association with MHC class II molecules of the DR, DQ or DP type. MHC Class II molecules are expressed by professional antigen presenting cells (APCs), such as macrophages and dendritic cells amongst others. Engagement of a MHC class II peptide complex by a cognate T-cell receptor on the surface of the T-cell, together with the cross-binding of certain other co-receptors such as the CD4 molecule, can induce an activated state within the T-cell. Activation leads to the release of cytokines further activating other lymphocytes such as B cells to produce antibodies or activating T killer cells as a full cellular immune response.

The ability of a peptide to bind a given MHC class II molecule for presentation on the surface of an APC is dependent on a number of factors most notably its primary sequence. This will influence both its propensity for proteolytic cleavage and also its affinity for binding within the peptide binding cleft of the MHC class II molecule. The MHC class II / peptide complex on the APC surface presents a binding face to a particular T-cell receptor (TCR) able to recognize determinants provided both by exposed residues of the peptide and the MHC class II molecule.

In the art there are procedures for identifying synthetic peptides able to bind MHC class II molecules (e.g. WO98/52976 and WO00/34317). Such peptides may not function as T-cell epitopes in all situations, particularly, *in vivo* due to the processing pathways or other phenomena. T-cell epitope identification is the first step to epitope elimination. The identification and removal of potential T-cell epitopes from proteins has been previously disclosed. In the art methods have been provided to enable the detection of T-cell epitopes usually by computational means scanning for recognized sequence motifs in experimentally determined T-cell epitopes or alternatively using computational techniques to predict MHC class II-binding peptides and in particular DR-binding peptides.
WO98/52976 and WO00/34317 teach computational threading approaches to identifying polypeptide sequences with the potential to bind a sub-set of human MHC class II DR allotypes. In these teachings, predicted T-cell epitopes are removed by the use of judicious amino acid substitution within the primary sequence of the therapeutic antibody or non-antibody protein of both non-human and human derivation.

Other techniques exploiting soluble complexes of recombinant MHC molecules in combination with synthetic peptides and able to bind to T-cell clones from peripheral blood samples from human or experimental animal subjects have been used in the art [Kern, F. et al (1998) *Nature Medicine* 4:975-978; Kwok, W.W. et al (2001) *TRENDS in Immunol.* 22:583-588]. These and other schemes including for example the use of whole FVIIIproteins or FVIII derived synthetic peptides or variant molecules thereof which are screened for molecules with altered ability to bind or stimulate T-cells may also be exploited in an epitope identification strategy.

As depicted above and as consequence thereof, it would be desirable to identify and to remove or at least to reduce T-cell epitopes from a given in principal therapeutically valuable but originally immunogenic peptide, polypeptide or protein.

One of these therapeutically valuable molecules is FVIII. The present invention provides for modified forms of human FVIII with one or more T cell epitopes removed. FVIII is a coagulation factor within the intrinsic pathway of blood coagulation. FVIII is a cofactor for factor IXa that, in the presence of calcium ions and phospholipid, converts factor X to the activated form Xa. The molecular genetics of FVIII are well studied not least as defects in the X-linked gene for FVIII give rise to hemophilia A. The FVIII gene encodes two alternatively spliced transcripts giving rise to the large glycoprotein FVIII isoform A and the smaller isoform B. In the native state multiple degradation or processed forms derived from the isoform A precursor can be identified and particular functional activities have been ascribed to particular fragments. FVIII protein may be functionally defined as a factor capable of correcting the coagulation defect in plasma derived from patients affected by hemophilia A.

For the treatment of hemophilia A, FVIII has been produced in purified form from human or porcine plasma and more recently by recombinant DNA technologies. Despite the availability of therapeutic quantities of FVIII, there is a continued need for FVIII analogues with enhanced properties. Desired enhancements include

alternative schemes and modalities for the expression and purification of the protein, but also and especially, improvements in the biological properties of the protein. There is a particular need for enhancement of the *in vivo* characteristics when administered to the human subject. In this regard, it is highly desired to provide FVIII with reduced or absent potential to induce an immune response in the human subject. Such proteins would expect to display an increased circulation time within the human subject and would be of particular benefit in chronic and recurring disease settings such as is the case hemophillia A.

It is a particular objective of the present invention to provide modified FVIII proteins in which the immune characteristic is modified by means of reduced numbers of potential T-cell epitopes.

Others have provided FVIII molecules and in particular recombinant modified FVIII [US, 4,757,006; US,5,633,150; US,5,668,108], but none of these teachings recognise the importance of T cell epitopes to the immunogenic properties of the protein nor have been conceived to directly influence said properties in a specific and controlled way according to the scheme of the present invention.

However, there is a continued need for FVIII analogues with enhanced properties. Desired enhancements include alternative schemes and modalities for the expression and purification of the said therapeutic, but also and especially, improvements in the biological properties of the protein. There is a particular need for enhancement of the *in vivo* characteristics when administered to the human subject. In this regard, it is highly desired to provide FVIII with reduced or absent potential to induce an immune response in the human subject.

SUMMARY AND DESCRIPTION OF THE INVENTION

The present invention provides for modified forms of human factor VIII, herein called "FVIII", in which the immune characteristic is modified by means of reduced or removed numbers of potential T-cell epitopes.

The invention discloses sequences identified within the FVIII primary sequence that are potential T-cell epitopes by virtue of MHC class II binding potential. This

disclosure specifically pertains the human FVIII protein being isoform A of 2332 amino acid residues and not including the native N-terminal signal sequence. The invention discloses also specific positions within the primary sequence of the molecule which according to the invention are to be altered by specific amino acid substitution, addition or deletion whilst retaining to a maximum degree the biological activity of the protein. In cases in which the loss of immunogenicity can be achieved only by a simultaneous loss of biological activity it is possible to restore said activity by further alterations within the amino acid sequence of the protein.

The invention furthermore discloses methods to produce such modified molecules, and above all methods to identify said T-cell epitopes which require alteration in order to reduce or remove immunogenic sites.

The present invention provides for modified forms of FVIII proteins that are expected to display enhanced properties *in vivo*. The present invention discloses the major regions of the FVIII primary sequence that are immunogenic in man and provides modification to the said sequences to eliminate or reduce the immunogenic effectiveness of these sites. In one embodiment, synthetic peptides comprising the said immunogenic regions can be provided in pharmaceutical composition for the purpose of promoting a tolerogenic response to the whole molecule. In a further embodiment, the modified FVIII molecules of the present invention can be used in pharmaceutical compositions.

In summary the invention relates to the following issues:

- a modified molecule having the biological activity of FVIII and being substantially non-immunogenic or less immunogenic than any non-modified molecule having the same biological activity when used *in vivo*;
- an accordingly specified molecule, wherein said loss of immunogenicity is achieved by removing one or more T-cell epitopes derived from the originally non-modified molecule;
- an accordingly specified molecule, wherein said loss of immunogenicity is achieved by reduction in numbers of MHC allotypes able to bind peptides derived from said molecule;
- an accordingly specified molecule, wherein one T-cell epitope is removed;

- an accordingly specified molecule, wherein said originally present T-cell epitopes are MHC class II ligands or peptide sequences which show the ability to stimulate or bind T-cells via presentation on class II;
- an accordingly specified molecule, wherein said peptide sequences are selected from the group as depicted in Table 1;
- an accordingly specified molecule, wherein 1 – 9 amino acid residues, preferably one amino acid residue in any of the originally present T-cell epitopes are altered;
- an accordingly specified molecule, wherein the alteration of the amino acid residues is substitution, addition or deletion of originally present amino acid(s) residue(s) by other amino acid residue(s) at specific position(s);
- an accordingly specified molecule, wherein, if necessary, additionally further alteration usually by substitution, addition or deletion of specific amino acid(s) is conducted to restore biological activity of said molecule;
- a peptide molecule of above sharing greater than 90% amino acid identity with any of the peptide sequences of Table 1;
- a peptide molecule of above sharing greater than 80% amino acid identity with any of the peptide sequences of Table 1;
- peptide sequences as above able to bind MHC class II;
- an accordingly specified FVIII molecule, wherein one or more of the amino acid substitutions is conducted at a position corresponding to any of the amino acids specified within Table 1
- a pharmaceutical composition comprising any of the peptides or modified peptides of above having the activity of binding to MHC class II
- a DNA sequence or molecule which codes for any of said specified modified molecules as defined above and below;
- a pharmaceutical composition comprising a modified molecule having the biological activity of FVIII
- a pharmaceutical composition as defined above and / or in the claims, optionally together with a pharmaceutically accepTable carrier, diluent or excipient;
- a method for manufacturing a modified molecule having the biological activity of FVIII as defined in any of the claims of the above-cited claims comprising

or part thereof; (ii) identifying one or more potential T-cell epitopes within the amino acid sequence of the protein by any method including determination of the binding of the peptides to MHC molecules using *in vitro* or *in silico* techniques or biological assays; (iii) designing new sequence variants with one or more amino acids within the identified potential T-cell epitopes modified in such a way to substantially reduce or eliminate the activity of the T-cell epitope as determined by the binding of the peptides to MHC molecules using *in vitro* or *in silico* techniques or biological assays; (iv) constructing such sequence variants by recombinant DNA techniques and testing said variants in order to identify one or more variants with desirable properties; and (v) optionally repeating steps (ii) – (iv);

- an accordingly specified method, wherein step (iii) is carried out by substitution, addition or deletion of 1 – 9 amino acid residues in any of the originally present T-cell epitopes;
- an accordingly specified method, wherein the alteration is made with reference to an homologous protein sequence and / or *in silico* modeling techniques;
- an accordingly specified method, wherein step (ii) of above is carried out by the following steps: (a) selecting a region of the peptide having a known amino acid residue sequence; (b) sequentially sampling overlapping amino acid residue segments of predetermined uniform size and constituted by at least three amino acid residues from the selected region; (c) calculating MHC Class II molecule binding score for each said sampled segment by summing assigned values for each hydrophobic amino acid residue side chain present in said sampled amino acid residue segment; and (d) identifying at least one of said segments suiTable for modification, based on the calculated MHC Class II molecule binding score for that segment, to change overall MHC Class II binding score for the peptide without substantially reducing therapeutic utility of the peptide; step (c) is preferably carried out by using a Böhm scoring function modified to include 12-6 van der Waal's ligand-protein energy repulsive term and ligand conformational energy term by (1) providing a first data base of MHC Class II molecule models; (2) providing a second data base of allowed peptide backbones for said MHC Class II molecule models; (3) selecting a model from said first data base; (4) selecting an allowed peptide backbone from said second data base; (5) identifying amino acid residue side chains

present in each sampled segment; (6) determining the binding affinity value for all side chains present in each sampled segment; and repeating steps (1) through (5) for each said model and each said backbone;

- a 13mer T-cell epitope peptide having a potential MHC class II binding activity and created from non-modified FVIII, selected from the group as depicted in Table 1 and its use for the manufacture of FVIII having substantially no or less immunogenicity than any non-modified molecule with the same biological activity when used *in vivo*;
- a peptide sequence consisting of at least 9 consecutive amino acid residues of a 13mer T-cell epitope peptide as specified above and its use for the manufacture of FVIII having substantially no or less immunogenicity than any non-modified molecule and having the biological activity of a human factor VIII when used *in vivo*;
- a 13mer T-cell epitope peptide having a potential MHC class II binding activity and created from non-modified FVIII, selected from any of the group of sequences (Table 1) and its use for the manufacture of FVIII having substantially no or less immunogenicity than any non-modified molecule and having the biological activity of a human factor VIII when used *in vivo*;
- a peptide sequence consisting of at least 9 consecutive amino acid residues of a 13mer T-cell epitope peptide as derived from any of the sequences in Table 1 and its use for the manufacture of FVIII having substantially no or less immunogenicity than any non-modified molecule and having the biological activity of a human factor VIII when used *in vivo*.

The term "T-cell epitope" means according to the understanding of this invention an amino acid sequence which is able to bind MHC class II, able to stimulate T-cells and / or also to bind (without necessarily measurably activating) T-cells in complex with MHC class II.

The term "peptide" as used herein and in the appended claims, is a compound that includes two or more amino acids. The amino acids are linked together by a peptide bond (defined herein below). There are 20 different naturally occurring amino acids involved in the biological production of peptides, and any number of them may be linked in any order to form a peptide chain or ring. The naturally occurring amino acids employed in the biological production of peptides often

the L-configuration. Synthetic peptides can be prepared employing conventional synthetic methods, utilizing L-amino acids, D-amino acids, or various combinations of amino acids of the two different configurations. Some peptides contain only a few amino acid units. Short peptides, e.g., having less than ten amino acid units, are sometimes referred to as "oligopeptides". Other peptides contain a large number of amino acid residues, e.g. up to 100 or more, and are referred to as "polypeptides". By convention, a "polypeptide" may be considered as any peptide chain containing three or more amino acids, whereas a "oligopeptide" is usually considered as a particular type of "short" polypeptide. Thus, as used herein, it is understood that any reference to a "polypeptide" also includes an oligopeptide. Further, any reference to a "peptide" includes polypeptides, oligopeptides, and proteins. Each different arrangement of amino acids forms different polypeptides or proteins. The number of polypeptides–and hence the number of different proteins–that can be formed is practically unlimited. "Alpha carbon (Cα)" is the carbon atom of the carbon-hydrogen (CH) component that is in the peptide chain. A "side chain" is a pendant group to Cα that can comprise a simple or complex group or moiety, having physical dimensions that can vary significantly compared to the dimensions of the peptide.
The invention may be applied to any FVIII species of molecule with substantially the same primary amino acid sequences as those disclosed herein and would include therefore FVIII molecules derived by genetic engineering means or other processes and may contain more or less than 2332 amino acid residues.
FVIII proteins such as identified from other mammalian sources have in common many of the peptide sequences of the present disclosure and have in common many peptide sequences with substantially the same sequence as those of the disclosed listing. Such protein sequences equally therefore fall under the scope of the present invention.

The invention is conceived to overcome the practical reality that soluble proteins introduced into autologous organisms can trigger an immune response resulting in development of host antibodies that bind to the soluble protein. A prominent example of this phenomenon amongst others, is the clinical use of interferon alpha 2 (INFα2). A significant proportion of human patients treated with INFα2 make antibodies despite the fact that this protein is produced endogenously

[Russo, D. et al (1996) *ibid*; Stein, R. et al (1988) *ibid*]. For many patients with hemophillia A antibody responses to the existing therapeutic preparations of FVIII is a significant problem in the mangement of their disease. In the absence of endogenous FVIII in their circulation, the therapeutic is recognised as a foriegn protein immunologically and stimulates a dose limiting immune response. The present invention seeks to address this by providing FVIIIproteins with altered propensity to elicit an immune response on administration to the human host. According to the methods described herein, the inventors have discovered the regions of the FVIII molecule comprising the critical T-cell epitopes driving the immune responses to this protein.

The general method of the present invention leading to the modified FVIII comprises the following steps:

(a) determining the amino acid sequence of the polypeptide or part thereof;

(b) identifying one or more potential T-cell epitopes within the amino acid sequence of the protein by any method including determination of the binding of the peptides to MHC molecules using *in vitro* or *in silico* techniques or biological assays;

(c) designing new sequence variants with one or more amino acids within the identified potential T-cell epitopes modified in such a way to substantially reduce or eliminate the activity of the T-cell epitope as determined by the binding of the peptides to MHC molecules using *in vitro* or *in silico* techniques or biological assays. Such sequence variants are created in such a way to avoid creation of new potential T-cell epitopes by the sequence variations unless such new potential T-cell epitopes are, in turn, modified in such a way to substantially reduce or eliminate the activity of the T-cell epitope; and

(d) constructing such sequence variants by recombinant DNA techniques and testing said variants in order to identify one or more variants with desirable properties according to well known recombinant techniques.

The identification of potential T-cell epitopes according to step (b) can be carried out according to methods describes previously in the prior art. SuiTable methods are disclosed in WO 98/59244; WO 98/52976; WO 00/34317 and may preferably

be used to identify binding propensity of FVIII-derived peptides to an MHC class II molecule.

In practice a number of variant FVIII proteins will be produced and tested for the desired immune and functional characteristic. The variant proteins will most preferably be produced by recombinant DNA techniques although other procedures including chemical synthesis of FVIII fragments may be contemplated.

The results of an analysis according to step (b) of the above scheme and pertaining to the human FVIII protein sequence is presented in TABLE 1.

The invention relates to FVIII analogues in which substitutions of at least one amino acid residue have been made at positions resulting in a substantial reduction in activity of or elimination of one or more potential T-cell epitopes from the protein. One or more amino acid substitutions at particular points within any of the potential MHC class II ligands identified in TABLE 1 may result in a FVIII molecule with a reduced immunogenic potential when administered as a therapeutic to the human host.

It is most preferred to provide an FVIII molecule in which amino acid modification (e.g. a substitution) is conducted within the most immunogenic regions of the parent molecule. The major preferred embodiments of the present invention comprise FVIII molecules for which any of the MHC class II ligands of TABLE 1 are altered such as to eliminate binding or otherwise reduce the numbers of MHC allotypes to which the peptide can bind.

For the elimination of T-cell epitopes, amino acid substitutions are preferably made at appropriate points within the peptide sequence predicted to achieve substantial reduction or elimination of the activity of the T-cell epitope. In practice an appropriate point will preferably equate to an amino acid residue binding within one of the pockets provided within the MHC class II binding groove.

It is most preferred to alter binding within the first pocket of the cleft at the so-called P1 or P1 anchor position of the peptide. The quality of binding interaction between the P1 anchor residue of the peptide and the first pocket of the MHC class II binding groove is recognized as being a major determinant of overall binding affinity for the whole peptide. An appropriate substitution at this position of the peptide will be for a residue less readily accommodated within the pocket, for example, substitution to a more hydrophilic residue. Amino acid residues in the peptide at positions equating to binding within other pocket regions within the MHC binding cleft are also considered and fall under the scope of the present.

It is understood that single amino acid substitutions within a given potential T-cell epitope are the most preferred route by which the epitope may be eliminated. Combinations of substitution within a single epitope may be contemplated and for example can be particularly appropriate where individually defined epitopes are in overlap with each other. Moreover, amino acid substitutions either singly within a given epitope or in combination within a single epitope may be made at positions not equating to the "pocket residues" with respect to the MHC class II binding groove, but at any point within the peptide sequence. Substitutions may be made with reference to an homologues structure or structural method produced using *in silico* techniques known in the art and may be based on known structural features of the molecule according to this invention. All such substitutions fall within the scope of the present invention.

Amino acid substitutions other than within the peptides identified above may be contemplated particularly when made in combination with substitution(s) made within a listed peptide. For example a change may be contemplated to restore structure or biological activity of the variant molecule. Such compensatory changes and changes to include deletion or addition of particular amino acid residues from the FVIII polypeptide resulting in a variant with desired activity and in combination with changes in any of the disclosed peptides fall under the scope of the present.

In as far as this invention relates to modified FVIII, compositions containing such modified FVIII protein or regions of modified FVIII and methods

compositions should be considered within the scope of the invention. In another aspect, the present invention relates to nucleic acids encoding modified FVIII entities. In a further aspect the present invention relates to methods for therapeutic treatment of humans using the modified FVIII proteins. In a further aspect still, the invention relates to methods for therapeutic treatment using pharmaceutical preparations comprising peptide or derivative molecules with sequence identity or part identity with the sequences herein disclosed.

The invention will now be illustrated by the following Table:

Table 1: provides a list of peptide sequences in human Factor VIII with potential human MHC class II binding activity Peptides are 13-mers, amino acids are identified using single letter code

TRRYYLGAVELSW,	RRYYLGAVELSWD,	RYYLGAVELSWDY,	YYLGAVELSWDYM,
GAVELSWDYMQSD,	VELSWDYMQSDLG,	LSWDYMQSDLGEL,	WDYMQSDLGELPV,
DYMQSDLGELPVD,	SDLGELPVDARFP,	GELPVDARFPPRV,	LPVDARFPPRVPK,
ARFPPRVPKSFPF,	PRVPKSFPFNTSV,	PKSFPFNTSVVYK,	KSFPFNTSVVYKK,
FPFNTSVVYKKTL,	TSVVYKKTLFVEF,	SVVYKKTLFVEFT,	VVYKKTLFVEFTD,
KTLFVEFTDHLFN,	TLFVEFTDHLFNI,	LFVEFTDHLFNIA,	VEFTDHLFNIAKP,
EFTDHLFNIAKPR,	DHLFNIAKPRPPW,	HLFNIAKPRPPWM,	FNIAKPRPPWMGL,
PPWMGLLGPTIQA,	PWMGLLGPTIQAE,	MGLLGPTIQAEVY,	GLLGPTIQAEVYD,
LLGPTIQAEVYDT,	PTIQAEVYDTVVI,	AEVYDTVVITLKN,	EVYDTVVITLKNM,
VYDTVVITLKNMA,	DTVVITLKNMASH,	TVVITLKNMASHP,	VVITLKNMASHPV,
ITLKNMASHPVSL,	TLKNMASHPVSLH,	KNMASHPVSLHAV,	SHPVSLHAVGVSY,
HPVSLHAVGVSYW,	PVSLHAVGVSYWK,	VSLHAVGVSYWKA,	HAVGVSYWKASEG,
VGVSYWKASEGAE,	VSYWKASEGAEYD,	SYWKASEGAEYDD,	EGAEYDDQTSQRE,
AEYDDQTSQREKE,	QREKEDDKVFPGG,	DKVFPGGSHTYVW,	KVFPGGSHTYVWQ,
GSHTYVWQVLKEN,	HTYVWQVLKENGP,	TYVWQVLKENGPM,	YVWQVLKENGPMA,
WQVLKENGPMASD,	QVLKENGPMASDP,	KENGPMASDPLCL,	GPMASDPLCLTYS,
DPLCLTYSYLSHV,	LCLTYSYLSHVDL,	LTYSYLSHVDLVK,	YSYLSHVDLVKDL,
SYLSHVDLVKDLN,	SHVDLVKDLNSGL,	VDLVKDLNSGLIG,	DLVKDLNSGLIGA,
KDLNSGLIGALLV,	DLNSGLIGALLVC,	LNSGLIGALLVCR,	SGLIGALLVCREG,
GLIGALLVCREGS,	IGALLVCREGSLA,	GALLVCREGSLAK,	ALLVCREGSLAKE,
LLVCREGSLAKEK,	GSLAKEKTQTLHK,	QTLHKFILLFAVF,	TLHKFILLFAVFD,
HKFILLFAVFDEG,	KFILLFAVFDEGK,	FILLFAVFDEGKS,	ILLFAVFDEGKSW,
LLFAVFDEGKSWH,	FAVFDEGKSWHSE,	AVFDEGKSWHSET,	KSWHSETKNSLMQ,

NSLMQDRDAASAR, SLMQDRDAASARA, MQDRDAASARAWP, RAWPKMHTVNGYV,
PKMHTVNGYVNRS, HTVNGYVNRSLPG, NGYVNRSLPGLIG, GYVNRSLPGLIGC,
RSLPGLIGCHRKS, PGLIGCHRKSVYW, GLIGCHRKSVYWH, KSVYWHVIGMGTT,
SVYWHVIGMGTTP, VYWHVIGMGTTPE, WHVIGMGTTPEVH, HVIGMGTTPEVHS,
IGMGTTPEVHSIF, GTTPEVHSIFLEG, PEVHSIFLEGHTF, HSIFLEGHTFLVR,
SIFLEGHTFLVRN, IFLEGHTFLVRNH, GHTFLVRNHRQAS, HTFLVRNHRQASL,
TFLVRNHRQASLE, FLVRNHRQASLEI, VRNHRQASLEISP, RQASLEISPITFL,
QASLEISPITFLT, ASLEISPITFLTA, LEISPITFLTAQT, ISPITFLTAQTLL,
SPITFLTAQTLLM, ITFLTAQTLLMDL, TFLTAQTLLMDLG, QTLLMDLGQFLLF,
TLLMDLGQFLLFC, LLMDLGQFLLFCH, MDLGQFLLFCHIS, DLGQFLLFCHISS,
GQFLLFCHISSHQ, QFLLFCHISSHQH, FLLFCHISSHQHD, LLFCHISSHQHDG,
CHISSHQHDGMEA, SSHQHDGMEAYVK, DGMEAYVKVDSCP, EAYVKVDSCPEEP,
AYVKVDSCPEEPQ, VKVDSCPEEPQLR, DSCPEEPQLRMKN, PQLRMKNNEEAED,
LRMKNNEEAEDYD, NEEAEDYDDDLTD, EDYDDDLTDSEMD, DDDLTDSEMDVVR,
DDLTDSEMDVVRF, SEMDVVRFDDDNS, MDVVRFDDDNSPS, DVVRFDDDNSPSF,
VVRFDDDNSPSFI, VRFDDDNSPSFIQ, DNSPSFIQIRSVA, PSFIQIRSVAKKH,
SFIQIRSVAKKHP, IQIRSVAKKHPKT, RSVAKKHPKTWVH, KTWVHYIAAEEED,
TWVHYIAAEEEDW, VHYIAAEEEDWDY, HYIAAEEEDWDYA, EDWDYAPLVLAPD,
WDYAPLVLAPDDR, APLVLAPDDRSYK, PLVLAPDDRSYKS, LVLAPDDRSYKSQ,
VLAPDDRSYKSQY, RSYKSQYLNNGPQ, YKSQYLNNGPQRI, SQYLNNGPQRIGR,
QYLNNGPQRIGRK, NGPQRIGRKYKKV, QRIGRKYKKVRFM, RKYKKVRFMAYTD,
KKVRFMAYTDETF, KVRFMAYTDETFK, VRFMAYTDETFKT, RFMAYTDETFKTR,
FMAYTDETFKTRE, MAYTDETFKTREA, YTDETFKTREAIQ, ETFKTREAIQHES,
FKTREAIQHESGI, TREAIQHESGILG, EAIQHESGILGPL, QHESGILGPLLYG,
SGILGPLLYGEVG, GILGPLLYGEVGD, GPLLYGEVGDTLL, PLLYGEVGDTLLI,
LLYGEVGDTLLII, GEVGDTLLIIFKN, DTLLIIFKNQASR, TLLIIFKNQASRP,
LLIIFKNQASRPY, LIIFKNQASRPYN, IIFKNQASRPYNI, SRPYNIYPHGITD,
RPYNIYPHGITDV, YNIYPHGITDVRP, NIYPHGITDVRPL, PHGITDVRPLYSR,
HGITDVRPLYSRR, TDVRPLYSRRLPK, RPLYSRRLPKGVK, PLYSRRLPKGVKH,
RRLPKGVKHLKDF, KGVKHLKDFPILP, KHLKDFPILPGEI, LKDFPILPGEIFK,
KDFPILPGEIFKY, FPILPGEIFKYKW, PILPGEIFKYKWT, GEIFKYKWTVTVE,
EIFKYKWTVTVED, FKYKWTVTVEDGP, YKWTVTVEDGPTK, WTVTVEDGPTKSD,
VTVEDGPTKSDPR, GPTKSDPRCLTRY, SDPRCLTRYYSSF, RCLTRYYSSFVNM,
TRYYSSFVNMERD, RYYSSFVNMERDL, SSFVNMERDLASG, SFVNMERDLASGL,
VNMERDLASGLIG, NMERDLASGLIGP, RDLASGLIGPLLI, DLASGLIGPLLIC,
LASGLIGPLLICY, SGLIGPLLICYKE, GLIGPLLICYKES, GPLLICYKESVDQ,
PLLICYKESVDQR, LLICYKESVDQRG, [illegible]

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LFSVFDENRSWYL, FSVFDENRSWYLT, SVFDENRSWYLTE, RSWYLTENIQRFL,
SWYLTENIQRFLP, WYLTENIQRFLPN, ENIQRFLPNPAGV, QRFLPNPAGVQLE,
RFLPNPAGVQLED, AGVQLEDPEFQAS, GVQLEDPEFQASN, VQLEDPEFQASNI,
QLEDPEFQASNIM, PEFQASNIMHSIN, SNIMHSINGYVFD, NIMHSINGYVFDS,
HSINGYVFDSLQL, INGYVFDSLQLSV, NGYVFDSLQLSVC, GYVFDSLQLSVCL,
YVFDSLQLSVCLH, VFDSLQLSVCLHE, DSLQLSVCLHEVA, LQLSVCLHEVAYW,
LSVCLHEVAYWYI, VCLHEVAYWYILS, HEVAYWYILSIGA, VAYWYILSIGAQT,
AYWYILSIGAQTD, YWYILSIGAQTDF, WYILSIGAQTDFL, YILSIGAQTDFLS,
ILSIGAQTDFLSV, LSIGAQTDFLSVF, ISAQTDFLSVFFS, TDFLSVFFSGYTF,
DFLSVFFSGYTFK, LSVFFSGYTFKHK, SVFFSGYTFKHKM, VFFSGYTFKHKMV,
FSGYTFKHKMVYE, SGYTFKHKMVYED, YTFKHKMVYEDTL, HKMVYEDTLTLFP,
KMVYEDTLTLFPF, MVYEDTLTLFPFS, DTLTLFPFSGETV, LTLFPFSGETVFM,
TLFPFSGETVFMS, FPFSGETVFMSME, SGETVFMSMENPG, ETVFMSMENPGLW,
TVFMSMENPGLWI, VFMSMENPGLWIL, MSMENPGLWILGC, PGLWILGCHNSDF,
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CHNSDFRNRGMTA, SDFRNRGMTALLK, RGMTALLKVSSCD, TALLKVSSCDKNT,
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EDSYEDISAYLLS, DSYEDISAYLLSK, EDISAYLLSKNNA, DISAYLLSKNNAI,
ISAYLLSKNNAIE, SAYLLSKNNAIEP, AYLLSKNNAIEPR, YLLSKNNAIEPRS,
SKNNAIEPRSFSQ, NAIEPRSFSQNSR, RSFSQNSRHPSTR, KQFNATTIPENDI,
TTIPENDIEKTDP, NDIEKTDPWFAHR, TDPWFAHRTPMPK, DPWFAHRTPMPKI,
PWFAHRTPMPKIQ, TPMPKIQNVSSSD, PKIQNVSSSDLLM, QNVSSSDLLMLLR,
NVSSSDLLMLLRQ, SDLLMLLRQSPTP, DLLMLLRQSPTPH, LLMLLRQSPTPHG,
LMLLRQSPTPHGL, MLLRQSPTPHGLS, HGLSLSDLQEAKY, GLSLSDLQEAKYE,
LSLSDLQEAKYET, SDLQEAKYETFSD, AKYETFSDDPSPG, ETFSDDPSPGAID,
PSPGAIDSNNSLS, SPGAIDSNNSLSE, GAIDSNNSLSEMT, NSLSEMTHFRPQL,
SEMTHFRPQLHHS, THFRPQLHHSGDM, PQLHHSGDMVFTP, HSGDMVFTPESGL,
GDMVFTPESGLQL, DMVFTPESGLQLR, MVFTPESGLQLRL, SGLQLRLNEKLGT,
LQLRLNEKLGTTA, LRLNEKLGTTAAT, RLNEKLGTTAATE, EKLGTTAATELKK,
KLGTTAATELKKL, TAATELKKLDFKV, TELKKLDFKVSST, ELKKLDFKVSSTS,
KKLDFKVSSTSNN, KLDFKVSSTSNNL, LDFKVSSTSNNLI, FKVSSTSNNLIST,
TSNNLISTIPSDN, NNLISTIPSDNLA, NLISTIPSDNLAA, STIPSDNLAAGTD,
SDNLAAGTDNTSS, DNLAAGTDNTSSL, LAAGTDNTSSLGP, DNTSSLGPPSMPV,
SSLGPPSMPVHYD, PSMPVHYDSQLDT, MPVHYDSQLDTTL, PVHYDSQLDTTLF,
VHYDSQLDTTLFG, HYDSQLDTTLFGK, SQLDTTLFGKKSS, TTLFGKKSSPLTE,
TLFGKKSSPLTES, SPLTESGGPLSLS, GPLSLSEENNDSK, LSLSEENNDSKLL,
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	SWGKNVSSTESGR,	KNVSSTESGRLFK,	GRLFKGKRAHGPA,	RLFKGKRAHGPAL,
	FKGKRAHGPALLT,	AHGPALLTKDNAL,	HGPALLTKDNALF,	PALLTKDNALFKV,
	ALLTKDNALFKVS,	DNALFKVSISLLK,	NALFKVSISLLKT,	ALFKVSISLLKTN,
5	FKVSISLLKTNKT,	KVSISLLKTNKTS,	VSISLLKTNKTSN,	SISLLKTNKTSNN,
	ISLLKTNKTSNNS,	SLLKTNKTSNNSA,	LLKTNKTSNNSAT,	KTSNNSATNRKTH,
	NRKTHIDGPSLLI,	THIDGPSLLIENS,	DGPSLLIENSPSV,	GPSLLIENSPSVW,
	PSLLIENSPSVWQ,	SLLIENSPSVWQN,	LLIENSPSVWQNI,	IENSPSVWQNILE,
	PSVWQNILESDTE,	SVWQNILESDTEF,	QNILESDTEFKKV,	NILESDTEFKKVT,
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	VTPLIHDRMLMDK,	TPLIHDRMLMDKN,	PLIHDRMLMDKNA,	IHDRMLMDKNATA,
	HDRMLMDKNATAL,	DRMLMDKNATALR,	RMLMDKNATALRL,	MLMDKNATALRLN,
	DKNATALRLNHMS,	NATALRLNHMSNK,	TALRLNHMSNKTT,	LRLNHMSNKTTSS,
	NHMSNKTTSSKNM,	KNATALRLNHMSN,	ALRLNHMSNKTTS,	SKNMEMVQQKKEG,
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	GPIPPDAQNPDMS,	PDAQNPDMSFFKM,	NPDMSFFKMLFLP,	PDMSFFKMLFLPE,
	MSFFKMLFLPESA,	SFFKMLFLPESAR,	FFKMLFLPESARW,	FKMLFLPESARWI,
	KMLFLPESARWIQ,	MLFLPESARWIQR,	LFLPESARWIQRT,	SARWIQRTHGKNS,
	ARWIQRTHGKNSL,	RWIQRTHGKNSLN,	IQRTHGKNSLNSG,	NSLNSGQGPSPKQ,
20	PKQLVSLGPEKSV,	KQLVSLGPEKSVE,	QLVSLGPEKSVEG,	LVSLGPEKSVEGQ,
	VSLGPEKSVEGQN,	LGPEKSVEGQNFL,	GPEKSVEGQNFLS,	KSVEGQNFLSEKN,
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	NKVVVGKGEFTKD,	KVVVGKGEFTKDV,	VVVGKGEFTKDVG,	KGEFTKDVGLKEM,
	GEFTKDVGLKEMV,	KDVGLKEMVFPSS,	VGLKEMVFPSSRN,	GLKEMVFPSSRNL,
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	SSRNLFLTNLDNL,	SRNLFLTNLDNLH,	RNLFLTNLDNLHE,	NLFLTNLDNLHEN,
	LFLTNLDNLHENN,	LTNLDNLHENNTH,	TNLDNLHENNTHN,	NLDNLHENNTHNQ,
	LDNLHENNTHNQE,	DNLHENNTHNQEK,	EKKIQEEIEKKET,	KKIQEEIEKKETL,
	QEEIEKKETLIQE,	EEIEKKETLIQEN,	IEKKETLIQENVV,	KKETLIQENVVLP,
30	ETLIQENVVLPQI,	TLIQENVVLPQIH,	IQENVVLPQIHTV,	ENVVLPQIHTVTG,
	NVVLPQIHTVTGT,	VVLPQIHTVTGTK,	LPQIHTVTGTKNF,	PQIHTVTGTKNFM,
	IHTVTGTKNFMKN,	HTVTGTKNFMKNL,	KNFMKNLFLLSTR,	NFMKNLFLLSTRQ,
	FMKNLFLLSTRQN,	MKNLFLLSTRQNV,	KNLFLLSTRQNVE,	NLFLLSTRQNVEG,
	LFLLSTRQNVEGS,	FLLSTRQNVEGSY,	RQNVEGSYDGAYA,	QNVEGSYDGAYAP,
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	YAPVLQDFRSLND,	APVLQDFRSLNDS,	PVLQDFRSLNDST,	LQDFRSLNDSTNR,
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	TSQQNFVTQRSKR,	QQNFVTQRSKRAL,	QNFVTQRSKRALK,	NFVTQRSKRALKQ,
	QRSKRALKQFRLP,	RALKQFRLPLEET,	KQFRLPLEETELE,	QFRLPLEETELEK,
5	FRLPLEETELEKR,	LPLEETELEKRII,	PLEETELEKRIIV,	LEETELEKRIIVD,
	TELEKRIIVDDTS,	EKRIIVDDTSTQW,	KRIIVDDTSTQWS,	RIIVDDTSTQWSK,
	IIVDDTSTQWSKN,	VDDTSTQWSKNMK,	TQWSKNMKHLTPS,	SKNMKHLTPSTLT,
	KNMKHLTPSTLTQ,	KHLTPSTLTQIDY,	PSTLTQIDYNEKE,	STLTQIDYNEKEK,
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	DCLTRSHSIPQAN,	SHSIPQANRSPLP,	HSIPQANRSPLPI,	RSPLPIAKVSSFP,
	SPLPIAKVSSFPS,	PLPIAKVSSFPSI,	LPIAKVSSFPSIR,	IAKVSSFPSIRPI,
	AKVSSFPSIRPIY,	VSSFPSIRPIYLT,	SSFPSIRPIYLTR,	FPSIRPIYLTRVL,
	PSIRPIYLTRVLF,	SIRPIYLTRVLFQ,	RPIYLTRVLFQDN,	PIYLTRVLFQDNS,
15	IYLTRVLFQDNSS,	LTRVLFQDNSSHL,	TRVLFQDNSSHLP,	RVLFQDNSSHLPA,
	VLFQDNSSHLPAA,	QDNSSHLPAASYR,	NSSHLPAASYRKK,	SSHLPAASYRKKD,
	SHLPAASYRKKDS,	AASYRKKDSGVQE,	ASYRKKDSGVQES,	KKDSGVQESSHFL,
	KDSGVQESSHFLQ,	DSGVQESSHFLQG,	SGVQESSHFLQGA,	VQESSHFLQGAKK,
	SHFLQGAKKNNLS,	HFLQGAKKNNLSL,	NNLSLAILTLEMT,	NLSLAILTLEMTG,
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	TLEMTGDQREVGS,	LEMTGDQREVGSL,	TGDQREVGSLGTS,	QREVGSLGTSATN,
	REVGSLGTSATNS,	VGSLGTSATNSVT,	GSLGTSATNSVTY,	KNNLSLAILTLEM,
	NSVTYKKVENTVL,	SVTYKKVENTVLP,	VTYKKVENTVLPK,	KKVENTVLPKPDL,
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	VHIYQKDLFPTET,	HIYQKDLFPTETS,	KDLFPTETSNGSP,	DLFPTETSNGSPG,
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30	GKVPFLRVATESS,	KVPFLRVATESSA,	VPFLRVATESSAK,	PFLRVATESSAKT,
	FLRVATESSAKTP,	LRVATESSAKTPS,	VATESSAKTPSKL,	SKLLDPLAWDNHY,
	KLLDPLAWDNHYG,	LLDPLAWDNHYGT,	LDPLAWDNHYGTQ,	DPLAWDNHYGTQI,
	PLAWDNHYGTQIP,	LAWDNHYGTQIPK,	DNHYGTQIPKEEW,	NHYGTQIPKEEWK,
	GTQIPKEEWKSQE,	TQIPKEEWKSQEK,	KEEWKSQEKSPEK,	EEWKSQEKSPEKT,
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	AFKKKDTILSLNA,	DTILSLNACESNH,	TILSLNACESNHA,	ILSLNACESNHAI,
	LSLNACESNHAIA,	HAIAAINEGQNKP,	AIAAINEGQNKPE,	IAAINEGQNKPEI,

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QNKPEIEVTWAKQ, PEIEVTWAKQGRT, EIEVTWAKQGRTE, IEVTWAKQGRTER,
EVTWAKQGRTERL, VTWAKQGRTERLC, TERLCSQNPPVLK, ERLCSQNPPVLKR,
NPPVLKRHQREIT, PPVLKRHQREITR, PVLKRHQREITRT, LKRHQREITRTTL,
KRHQREITRTTLQ, REITRTTLQSDQE, TTLQSDQEEIDYD, QEEIDYDDTISVE,
EEIDYDDTISVEM, EIDYDDTISVEMK, IDYDDTISVEMKK, DTISVEMKKEDFD,
TISVEMKKEDFDI, ISVEMKKEDFDIY, SVEMKKEDFDIYD, VEMKKEDFDIYDE,
EDFDIYDEDENQS, DFDIYDEDENQSP, FDIYDEDENQSPR, DIYDEDENQSPRS,
YDEDENQSPRSFQ, PRSFQKKTRHYFI, RSFQKKTRHYFIA, TRHYFIAAVERLW,
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WDYGMSSSPHVLR, YGMSSSPHVLRNR, MSSSPHVLRNRAQ, SSSPHVLRNRAQS,
PHVLRNRAQSGSV, HVLRNRAQSGSVP, RAQSGSVPQFKKV, QSGSVPQFKKVVF,
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SSLISYEEDQRQG, SLISYEEDQRQGA, LISYEEDQRQGAE, ISYEEDQRQGAEP,
KNFVKPNETKTYF, NFVKPNETKTYFW, KTYFWKVQHHMAP, TYFWKVQHHMAPT,
YFWKVQHHMAPTK, WKVQHHMAPTKDE, HHMAPTKDEFDCK, TKDEFDCKAWAYF,
DEFDCKAWAYFSD, KAWAYFSDVDLEK, WAYFSDVDLEKDV, AYFSDVDLEKDVH,
SDVDLEKDVHSGL, DVDLEKDVHSGLI, VDLEKDVHSGLIG, LEKDVHSGLIGPL,
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GLIGPLLVCHTNT, IGPLLVCHTNTLN, GPLLVCHTNTLNP, PLLVCHTNTLNPA,
LLVCHTNTLNPAH, NTLNPAHGRQVTV, AHGRQVTVQEFAL, RQVTVQEFALFFT,
VTVQEFALFFTIF, TVQEFALFFTIFD, QEFALFFTIFDET, FALFFTIFDETKS,
ALFFTIFDETKSW, LFFTIFDETKSWY, FTIFDETKSWYFT, TIFDETKSWYFTE,
KSWYFTENMERNC, SWYFTENMERNCR, WYFTENMERNCRA, FTENMERNCRAPC,
ENMERNCRAPCNI, PCNIQMEDPTFKE, CNIQMEDPTFKEN, IQMEDPTFKENYR,
PTFKENYRFHAIN, ENYRFHAINGYIM, NYRFHAINGYIMD, YRFHAINGYIMDT,
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[illegible]

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YKMALYNLYPGVF, MALYNLYPGVFET, ALYNLYPGVFETV, YNLYPGVFETVEM,
NLYPGVFETVEML, PGVFETVEMLPSK, GVFETVEMLPSKA, FETVEMLPSKAGI,
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AGIWRVECLIGEH, GIWRVECLIGEHL, WRVECLIGEHLHA, ECLIGEHLHAGMS,
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STLFLVYSNKCQT, TLFLVYSNKCQTP, LFLVYSNKCQTPL, FLVYSNKCQTPLG,
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LGMASGHIRDFQI, SGHIRDFQITASG, GHIRDFQITASGQ, IRDFQITASGQYG,
RDFQITASGQYGQ, FQITASGQYGQWA, EHLHAGMSTLFLV, HLHAGMSTLFLVY,
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FLVYSNKCQTPLG, LVYSNKCQTPLGM, YSNKCQTPLGMAS, QTPLGMASGHIRD,
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LMVFFGNVDSSGI, MVFFGNVDSSGIK, VFFGNVDSSGIKH, FFGNVDSSGIKHN,
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IKHNIFNPPIIAR, KHNIFNPPIIARY, HNIFNPPIIARYI, NIFNPPIIARYIR,
NPPIIARYIRLHP, PPIIARYIRLHPT, PIIARYIRLHPTH, ARYIRLHPTHYSI,
RYIRLHPTHYSIR, IRLHPTHYSIRST, THYSIRSTLRMEL, YSIRSTLRMELMG,
STLRMELMGCDLN, TLRMELMGCDLNS, LRMELMGCDLNSC, MELMGCDLNSCSM,
ELMGCDLNSCSMP, LMGCDLNSCSMPL, CDLNSCSMPLGME, CSMPLGMESKAIS,
SMPLGMESKAISD, MPLGMESKAISDA, LGMESKAISDAQI, GMESKAISDAQIT,
SKAISDAQITASS, KAISDAQITASSY, AISDAQITASSYF, DAQITASSYFTNM,
AQITASSYFTNMF, TASSYFTNMFATW, SSYFTNMFATWSP, SYFTNMFATWSPS,
TNMFATWSPSKAR, NMFATWSPSKARL, FATWSPSKARLHL, ATWSPSKARLHLQ,

PSKARLHLQGRSN, ARLHLQGRSNAWR, LHLQGRSNAWRPQ, QGRSNAWRPQVNN,
NAWRPQVNNPKEW, PQVNNPKEWLQVD, NNPKEWLQVDFQK, KEWLQVDFQKTMK,
EWLQVDFQKTMKV, LQVDFQKTMKVTG, VDFQKTMKVTGVT, KTMKVTGVTTQGV,
MKVTGVTTQGVKS, TGVTTQGVKSLLT, VTTQGVKSLLTSM, QGVKSLLTSMYVK,
GVKSLLTSMYVKE, KSLLTSMYVKEFL, SLLTSMYVKEFLI, LTSMYVKEFLISS,
TSMYVKEFLISSS, SMYVKEFLISSSQ, MYVKEFLISSSQD, YVKEFLISSSQDG,
KEFLISSSQDGHQ, EFLISSSQDGHQW, FLISSSQDGHQWT, ISSSQDGHQWTLF,
SQDGHQWTLFFQN, GHQWTLFFQNGKV, HQWTLFFQNGKVK, WTLFFQNGKVKVF,
TLFFQNGKVKVFQ, LFFQNGKVKVFQG, NGKVKVFQGNQDS, GKVKVFQGNQDSF,
VKVFQGNQDSFTP, KVFQGNQDSFTPV, DSFTPVVNSLDPP, TPVVNSLDPPLLT,
PVVNSLDPPLLTR, NSLDPPLLTRYLR, SLDPPLLTRYLRI, PPLLTRYLRIHPQ,
PLLTRYLRIHPQS, TRYLRIHPQSWVH, RYLRIHPQSWVHQ, LRIHPQSWVHQIA,
HPQSWVHQIALRM, QSWVHQIALRMEV, SWVHQIALRMEVL, WVHQIALRMEVLG,
HQIALRMEVLGCE, IALRMEVLGCEAQ, LRMEVLGCEAQDL,

Patent Claims:

1. A protein having the biological activity of human Factor VIII (FVIII) and being substantially non-immunogenic or less immunogenic than any non-modified protein having the same biological activity when used in vivo.

2. A protein of claim 1 wherein said loss of immunogenicity is deduced to a reduced number of potential T-cell epitopes.

3. An amino acid sequence which is part of the sequence of an immunogenically non-modified Factor VIII molecule and has a potential MHC class II binding activity, selected from one of the 13mer epitopes as specified in Table 1.

4. A protein of any of the claims 1 – 2, wherein one or more of the amino acid sequences according to claim 3 were modified by substituting, inserting or deleting one or more amino acids within said amino acid sequences.

5. A pharmaceutical composition comprising a protein as defined in claim 4.

6. A method for manufacturing a protein of claim 4 comprising the following steps:
(I) determining the amino acid sequence of the polypeptide or part thereof.
(Ii) identifying one or more potential T cell epitopes within the amino acid sequence of the protein by any method including determination of the binding of the peptides to MHC molecules using *in vitro* or *in silico* techniques or biological assays;
(Iii) designing new sequence variants with one or more amino acids within the identified potential T cell epitopes modified in such a way to substantially reduce or eliminate the activity of the T cell epitope as determined by the binding of the peptides to MHC molecules using *in vitro* or *in silico* techniques or biological assays, said sequence variants are created in such a way to avoid creation of new potential T cell epitopes by the sequence variations unless such new potential T cell epitopes are, in

turn, modified in such a way to substantially reduce or eliminate the activity of the T cell epitope.

(iv) constructing such sequence variants by recombinant DNA techniques and testing said variants in order to identify one or more variants with desirable properties.

Abstract:

The present invention relates to polypeptides to be administered especially to humans and in particular for therapeutic use. The polypeptides are modified polypeptides whereby the modification results in a reduced propensity for the polypeptide to elicit an immune response upon administration to the human subject. The invention in particular relates to the modification of human Factor VIII (FVIII) to result in FVIII proteins that are substantially non-immunogenic or less immunogenic than any non-modified counterpart when used *in vivo*.